Triplet Repeat Instability in Human iPSCs

Grant Award Details

Triplet Repeat Instability in Human iPSCs

Grant Type: Basic Biology III

Grant Number: RB3-05022

Project Objective: The PI is studying the molecular basis of triplet repeat expansion/instability. Specifically, this project will determine the role of transcription and heterochromatin in expansion of triplet repeats during the establishment and propagation of iPSCs.

Investigator:

Name:	Joel Gottesfeld
Institution:	Scripps Research Institute
Type:	PI

Disease Focus: Huntington's Disease, Neurological Disorders

Human Stem Cell Use: iPS Cell

Cell Line Generation: iPS Cell

Award Value: $1,705,494

Status: Closed

Progress Reports

Reporting Period: Year 1

View Report

Reporting Period: Year 2

View Report

Reporting Period: Year 3

View Report

Grant Application Details

Application Title: Triplet Repeat Instability in Human iPSCs

Public Abstract: Over twenty human genetic diseases are caused by expansion of simple DNA sequences composed of repeats of three nucleotides (such as CAG, CTG, CGG and GAA) within essential genes. These repeats can occur within the region of a gene that encodes the protein, generally resulting in proteins with large stretches of repeats of just one amino acid, such as runs of glutamine. These proteins are toxic, cause the death of specific types of brain cells and result in diseases such as Huntington's disease (HD) and many of the spinocerebellar ataxias (a type of movement disorder). Other repeats can be in regions of genes that do not code for the protein itself, but are copied into messenger RNA, which is a copy of the gene that serves to generate the protein. These RNAs with expanded repeats are also toxic to cells, and sometimes these RNAs sequester essential cellular proteins. One example of this type of disease is Myotonic Dystrophy type 1, a form of muscular dystrophy. Lastly, there are two examples of repeat disorders where the repeats silence the genes harboring these mutations: these are Friedreich's ataxia (FRDA) and Fragile X syndrome (FXS). One limitation in the development of drugs to treat these diseases is the lack of appropriate cell models that represent the types of cells that are affected in these human diseases. With the advent of the technology to produce induced pluripotent stem cells from patient skin cells, and our ability to turn iPSCs into any cell type, such as neurons (brain cells) that are affected in these triplet repeat diseases, such cellular models are now becoming available. Our laboratories have generated iPSCs from fibroblasts obtained from patients with HD, FXS and FRDA. By comparing cells before and after reprogramming, we found that triplet repeats were expanded in the FRDA iPSCs, but not in HD iPSCs. This application is aimed at the understanding the molecular basis underlying triplet repeat expansion/instability that we have observed during the establishment and propagation of iPSCs from disease-specific fibroblasts. While artificial systems with reporter gene constructs have reproduced triplet repeat expansion in bacteria, yeast and mammalian cells, no cellular models have previously been reported that recapitulate repeat expansions at the endogenous cellular genes involved in these diseases. Therefore, our observations that repeat expansion is found in FRDA iPSCs provides the first opportunity to dissect the mechanisms involved in expansion at the molecular level for the authentic cellular genes in their natural chromatin environment. Repeat expansion is the central basis for these diseases, no matter what the outcome of the expansion (toxic protein or RNA or gene silencing), and a fuller understanding of how repeats expand may lead to new drugs to treat these diseases.

Statement of Benefit to California:	A major obstacle in the development of new drugs for human diseases is our lack of cell models that represent the tissues or organs that are affected in these diseases. Examples of such diseases are the triplet-repeat neurodegenerative diseases, such as Huntington's disease, the spinocerebellar ataxias, forms of muscular dystrophy, Fragile X syndrome and Friederich's ataxia. These diseases, although relatively rare compared to cancer or heart disease, affect thousands of individuals in California. Recent advances in stem cell biology now make it possible to generate cells that reflect the cell types at risk in these diseases (such as brain, heart and muscle cells), starting from patient skin cells. Skin cells can be turned into stem cell-like cells (induced pluripotent stem cells or iPSCs), which can then give rise to just about any cell type in the human body. During the course of our studies, we found that iPSCs derived from Friedreich's ataxia patient skin cells mimic the behavior of the genetic mutation in this disease. A simple repeat of the DNA sequence GAA is found in the gene encoding an essential protein called frataxin, and this repeat increases in length between generations in human families carrying this mutation. Over a certain threshold, the repeats silence this gene. It is also known that the repeats expand in brain cells in individuals with this disease. With the advent of patient derived iPSCs and neurons, we now have human model systems in which to study the mechanisms responsible for repeat expansion. We have already identified one set of proteins involved in repeat expansion and we now wish to delve more deeply into how the repeats expand. In this way, we may be able to identify new targets for drug development. We will extend our studies to Huntington's disease and Fragile X syndrome. We have identified two possible therapeutic approaches for Friedreich's ataxia, and identified molecules that either reactivate the silent gene or block repeat expansion. Our studies in related diseases may provide possible therapeutic strategies for these other disorders as well, which will be of benefit to patients suffering from these diseases, both in California and world-wide.